The Safety of AQUI-S® to Freshwater Finfish

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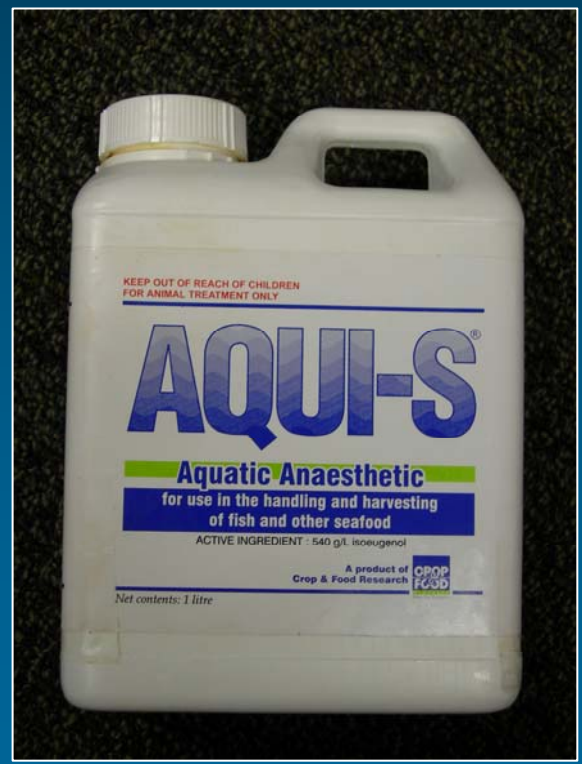
U.S. Fish & Wildlife Service

AADAP Program
Bozeman, MT

AQUI-S® Aquatic Anesthetic

- Fish anesthetic developed by
  AQUI-S New Zealand, Ltd.
- Synthetic
  - (50% isoeugenol)
- Approved in New Zealand, Australia, & Chile for use on food fish (no withdrawal period required)
- Candidate for FDA approval as “zero-withdrawal” anesthetic



Data Packages

→ Product Chemistry

→ Human Safety

→ Environmental Safety

→ Labeling and All Other Information

→ Efficacy (AADAP Program)

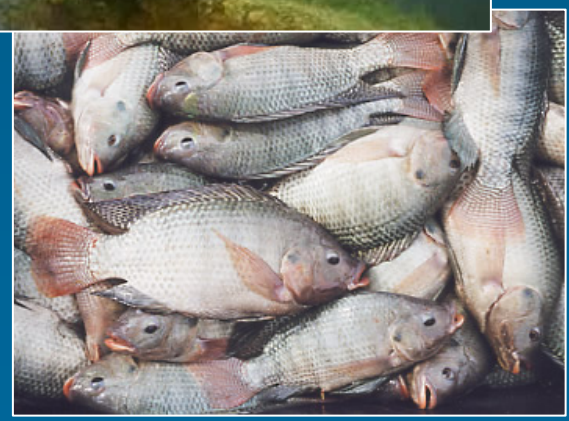
→ Target Animal Safety (AADAP Program)

Proposed Label Claim

Use AQUI-S® at 20 – 40 mg/L to sedate all freshwater salmonids for management and handling purposes.

Use AQUI-S® at 20 – 60 mg/L to sedate all freshwater coolwater finfish for management and handling purposes.

Use AQUI-S® at 40 – 60 mg/L to sedate all freshwater warmwater finfish for management and handling purposes.



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Introduction

- Target Animal Safety (TAS) studies must show an "adequate Margin of Safety"

- TAS studies with Therapeutic Drugs:
  - Margin of Safety = Concentration (3x, 5x, or 10x higher than highest efficacious concentration listed on the product label)

- TAS studies with Anesthetics (AQUI-S®):
  - Margin of Safety = Duration of exposure at a given concentration (e.g., Y minutes at X mg/L)

Objectives

- Determine longest exposure durations (min) at which survival was >95% to most sensitive life-stage of test fish (salmonids)

- Determine the Margin of Safety

Test Article and Test Species

- **AQUI-S®**
  - 0, 40, and 80 mg/L
- **Species**
  - Rainbow trout
  - Cutthroat trout
- **Life-stage**
  - small fingerling (1.5 in.)
- **Water temp**
  - 13 – 15°C

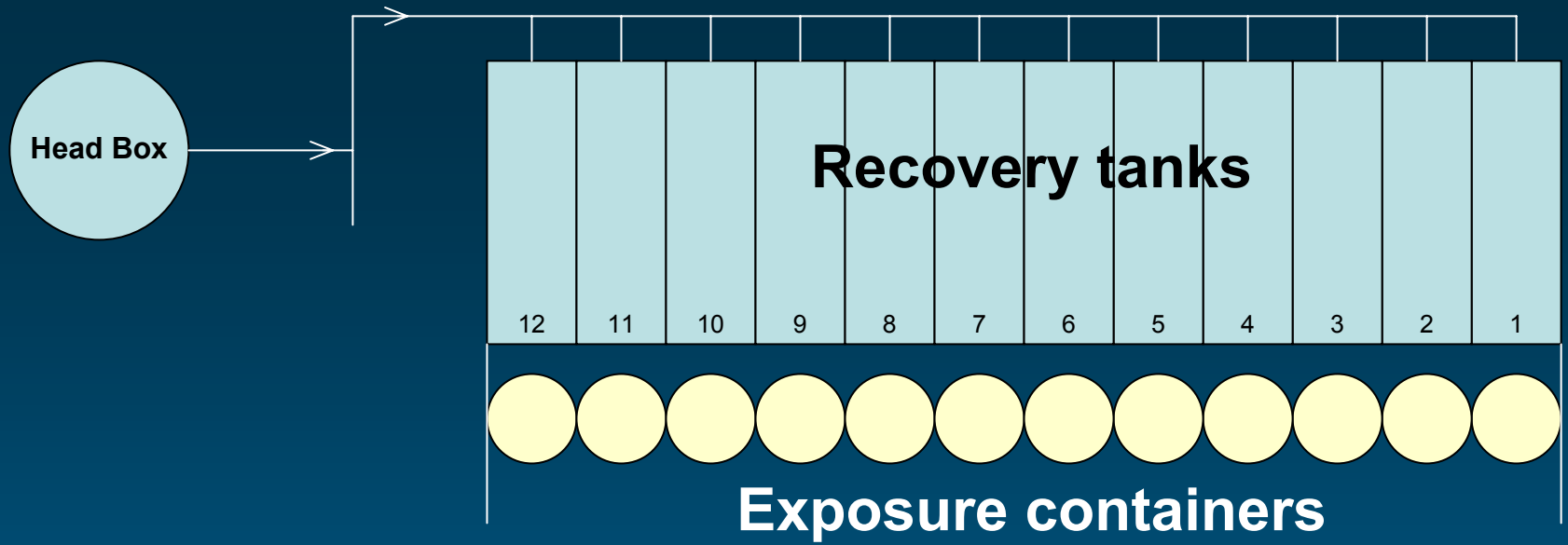
Study Design

Cutthroat Trout

Exposure duration (min)	Exposure concentration (mg/L)		
	0	**40**	**80**
T1	8.0	8.0	2.5
T2	9.8	9.8	3.0
T3	10.5	10.5	4.0
T4	12.0	12.0	4.8

- **Exposed 4 groups of test fish (n = 20 fish/group) at each of the 12 exposure regimens (concentration x duration)**

- **One "exposure/recovery replicate" = (exposure event) + (96 hr post-exposure "recovery" period)**



One
Exposure / Recovery
Replicate

AQUI-S® bulk solutions

- 0 mg/L
- 40 mg/L
- 80 mg/L

Tank Room

Study Conduct

- Study protocol (FDA approved)
- Good Lab Practice Compliant
  - (QA inspection)
- Blinding
- Randomization
- Dose verification

Exposure

And

Recovery

Pre - Exposure


Start exposure


Expose for T1, T2, T3, or T4


Start recovery period


End recovery period

Exposure Data

Recovery Data

Results for Cutthroat Trout

Exposure duration (min)	Mean Percent Survival at each Exposure Concentration (mg/L)		
	0	**40**	**80**
T1	100% **8.0 min**	100% **8.0 min**	100% **2.5 min**
T2	100% **9.8 min**	85% **9.8 min**	99% **3.0 min**
T3	100% **10.5 min**	88% **10.5 min**	80% **4.0 min**
T4	100% **12.0 min**	80% **12.0 min**	44% **4.8 min**

- **Time to handleable at 40 mg/L = 2.2 min (CVM, 2.2 min x 3 = 6.6 min)**

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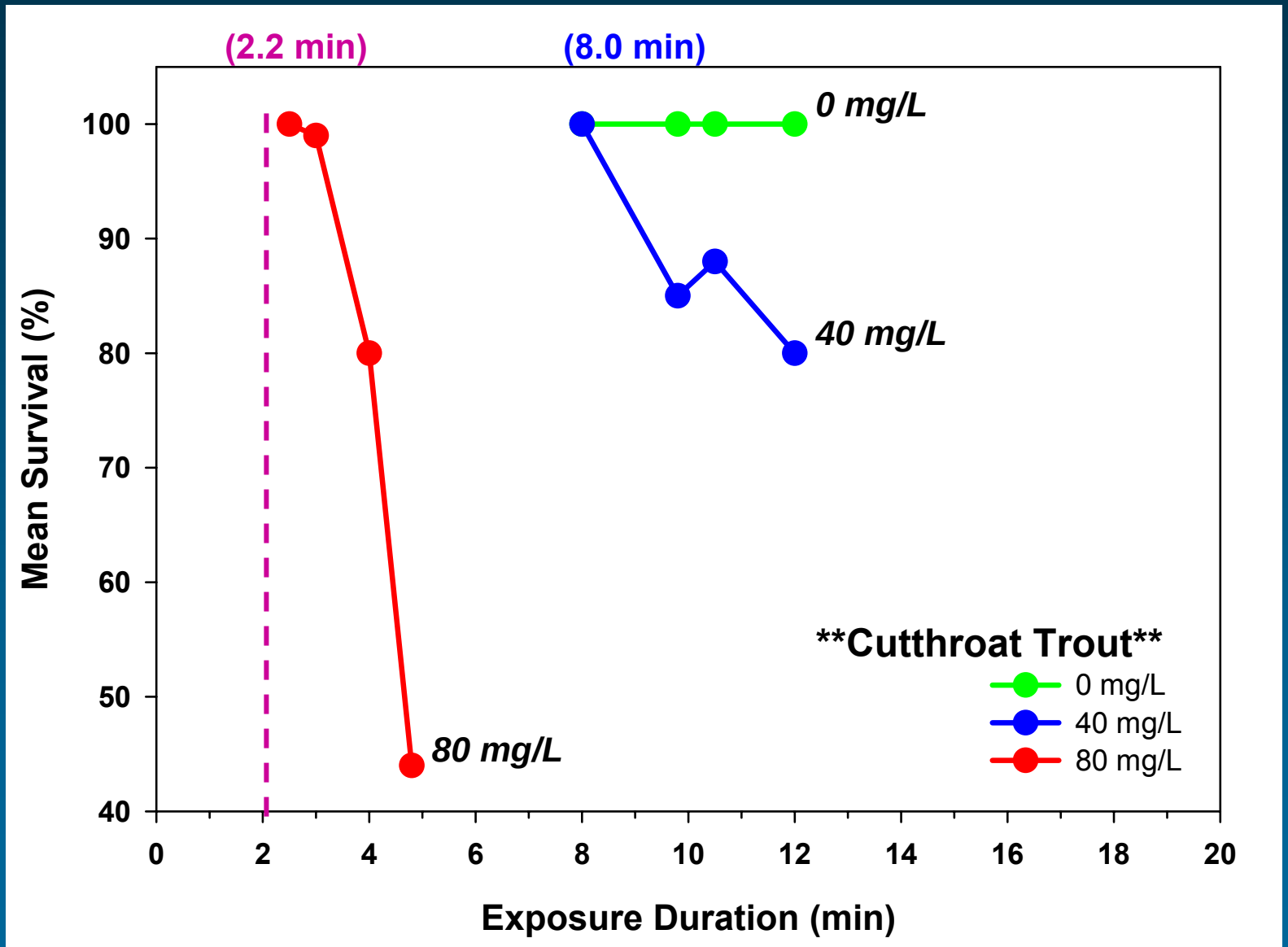
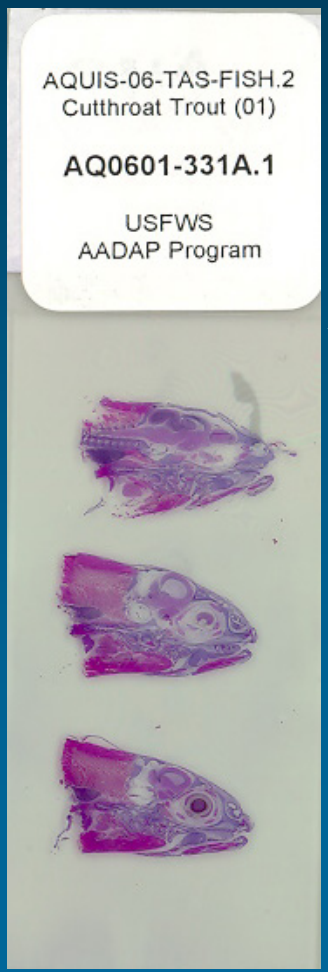
Results – Mean Percent Survival



Histology

Sectioned left-half of head to evaluate:
gills, brain, heart, eye, liver, and anterior kidney



Histology Slides

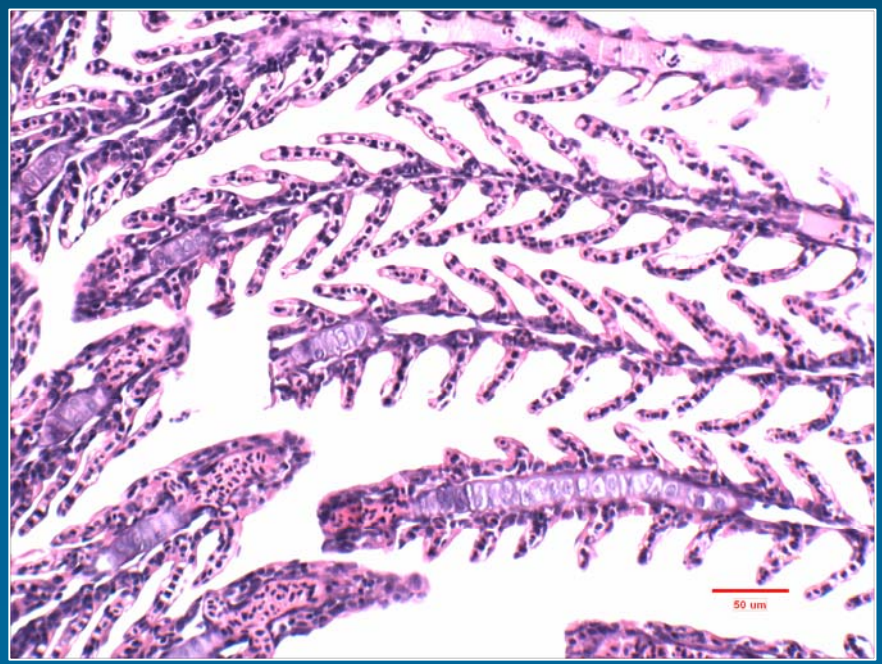
Sectioned left-half of body to evaluate:
posterior kidney, stomach, pyloric and rectal intestines, spleen, muscle, and skin

Histology Results

The only histological effects considered to be test-article induced and to be "safety concerns" were <u>scattered</u>

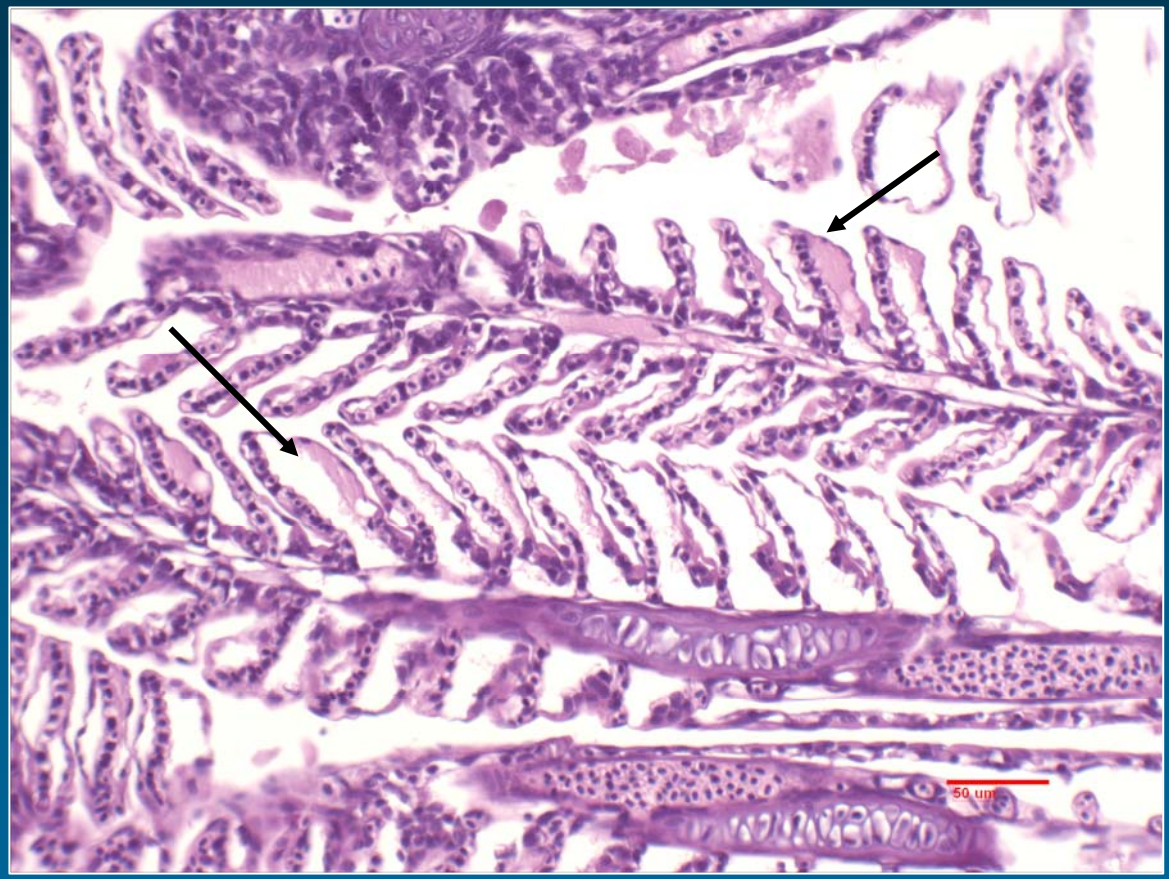
(1) epithelial separation in gill tissue (mild to severe)
and

(2) hypertrophy of gill epithelium (mild to severe)



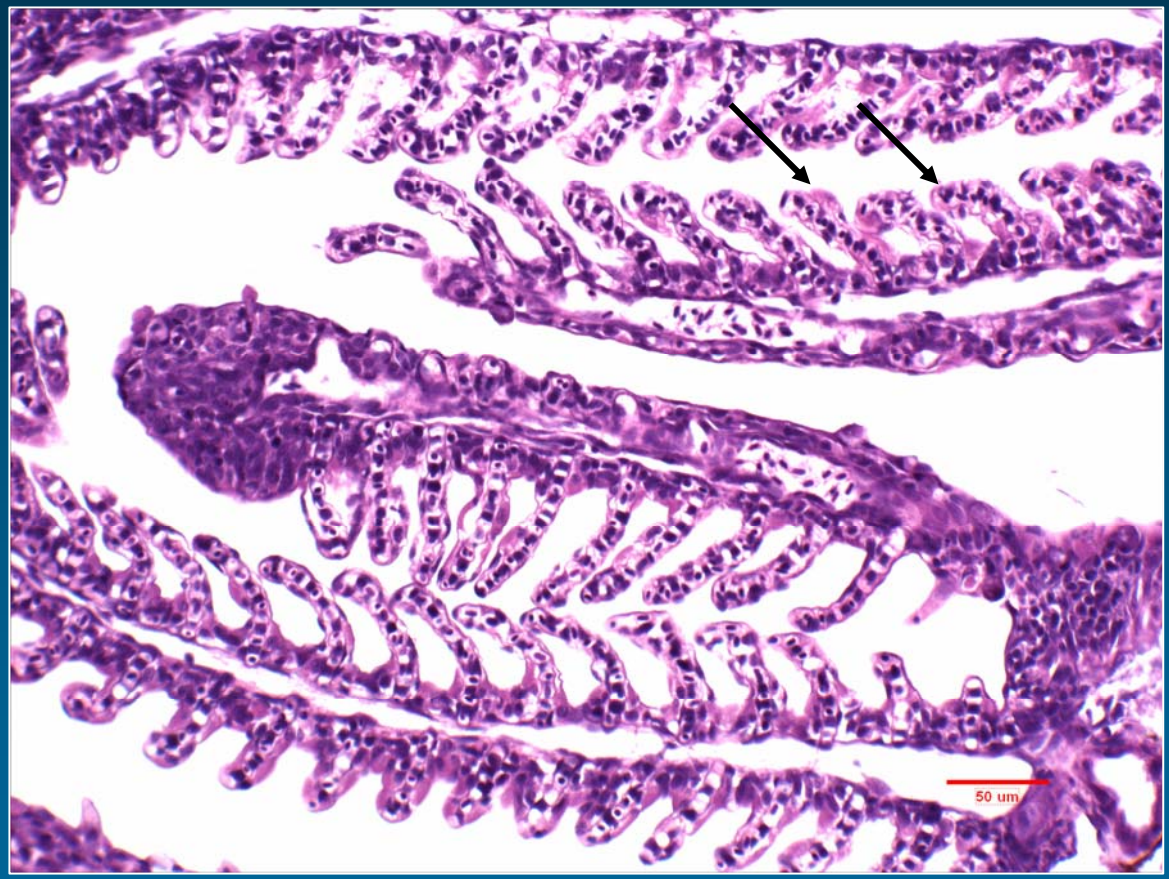
Normal gill tissue, 20x objective

Histology Results



<u>Epithelial separation</u> in gill lamellae of a fish from the $T3_{40\ mg/L}$ group. Note the plasma accumulation (arrows). (Sampled live; 20x objective)

Histology Results



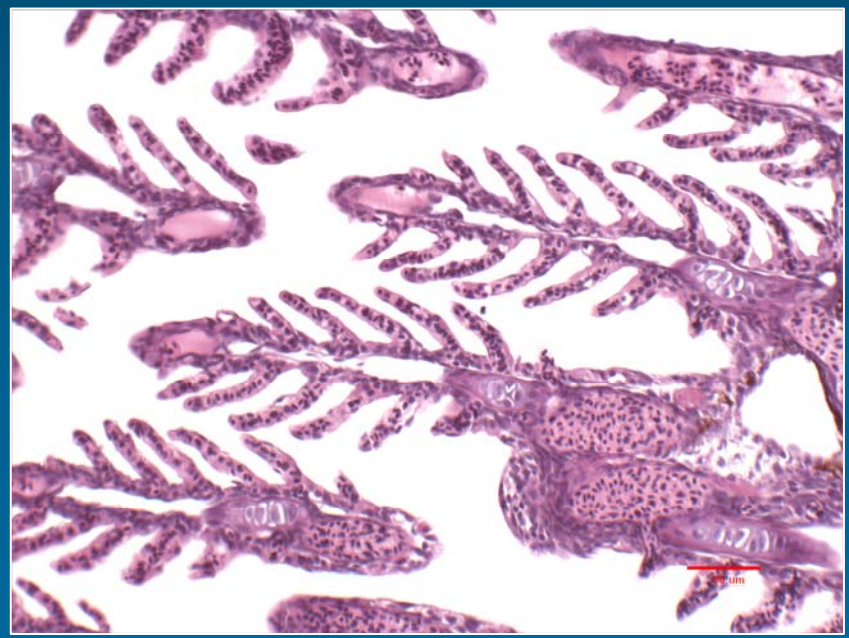
<u>Hypertrophy</u> of lamellar epithelium (arrows) in a fish from the $T3_{80\ mg/L}$ group. (Sampled live; 20x objective)

Conclusions

HISTOLOGY

CVM stated in a response letter from the rainbow trout TAS study that

“. . . no lesions were determined to be clinically significant. . . .”



Normal gill tissue, 20x objective

Conclusions

- Estimated exposure duration Margins of Safety

AQUI-S® Concentration	Margin of Safety
Rainbow Trout	
40 mg/L	4.5 min
Cutthroat Trout	
40 mg/L	5.8 min

Margin of Safety = (Longest time with >95% survival) – (Time to handleable)

- There is an adequate Margin of Safety associated with 40 mg/L AQUI-S® when sedating freshwater salmonids.

Last but Not Least . . .

Rainbow Trout Study

40 mg/L AQUI-S® was accepted as the highest effective and safe concentration for use on freshwater rainbow trout by CVM (December 2006)

(we anticipate acceptance of the Cutthroat trout safety study – to be submitted Spring 2007)

Any Questions ?

Aquatic Animal Drug Approval Partnership (AADAP) Program
www.fws.gov/fisheries/aadap